ZOOLOGICAL SCIENCE 6: 777–788 (1989)

Age-Related Changes and Sex Difference in the Ultrastructure of Renal Glomerulus in Wistar/Tw Rats

Win Win Yee, Sumio Takahashi
and Seiichiro Kawashima[1]

Zoological Institute, Faculty of Science, Hiroshima University, Hiroshima 730, and
[1]Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113, Japan

ABSTRACT—Changes in the renal glomerulus were electron microscopically examined in male and female rats of the Wistar/Tw strain at 1, 3, 6, 12 and 18 months of age. Initial lesions as focal thickening of the glomerular capillary basement membrane (GBM) and the fusion of glomerular epithelial cell foot processes (EpF) were encountered at 3 months in male and at 6 months in female rats. Twelve-month-old female rats showed focal thickening of the GBM with fusion of the EpF and the formation of vacuoles. At 18 months focal thickening of the GBM became segmental with fusion of the EpF and frequent occurrence of fusion of the mesangial matrix to the GBM. At 12 and 18 months, there were striking sex differences in the severity of the renal lesions. Only 12- and 18-month-old male rats showed extensive thickening of the GBM with nodular folds intermingled with massive mesangial matrix, extensive fusion, denudation of the EpF and degeneration of the epithelial cells.

INTRODUCTION

A marked increase in the water intake and urinary output with low electrolyte concentration was commonly observed in male rats of the Wistar/Tw strain maintained in our laboratories at the age of over 16 months [1–3]. These changes are correlated with histopathological changes of the kidney with about 70% of glomeruli degeneration [4]. We have recently light microscopically observed that 50% of the glomeruli was affected as early as at 3 months of age in male rats and more than 80% of the glomeruli, at 13 months. In contrast, in female rats the affected glomeruli were only about 40% at 9 months of age [5]. The retarded development of kidney lesions in female rats is well reflected by the changes in renal functions, that is, female rats of the Wistar/Tw strain develop polydipsia and polyuria at 19 months of age [6].

In electron microscopical study, we found degenerative changes in the epithelial cell cytoplasm as bearing slender processes, extreme enlargement of the mesangium intermingled with glomerular basement membrane (GBM) and the cortical collecting tubules filled with electron dense material and with completely flattened epithelial cells in male rats at 18 months of age, while the renal structure of 3-month-old rats was mostly healthy. At 3 months a slight thickening of the GBM with some fusion of the epithelial cell foot processes (EpF) was present in a few scattered areas of the GBM, but the cortical collecting tubules were normal [7]. Several authors have already reported the age-related changes in other strains of rats characterized by the thickening of the GBM and the enlargement of mesangium area of male rats [8–10]. Our results are generally in harmony with theirs. However, as clear sex difference in renal histopathological changes was the feature of the Wistar/Tw strain, we attempted to extend our electron microscopical study focusing on the male-female difference of the glomerulus as a function of age. The understanding of the factors causing this sex difference may elucidate the aging processes of the kidney structure and function.

Accepted November 14, 1988
Received July 11, 1988
[1] To whom reprint requests should be addressed.

MATERIALS AND METHODS

Male and female rats of the Wistar/Tw strain were used in the present study. They were maintained in a temperature- and light-controlled room with free access to laboratory chow (CA-1, Japan Clea Inc.) and tap water. At 1, 3, 6, 12 and 18 months of age, five male and five female rats each were killed by decapitation. Both kidneys were quickly removed and weighed. Frontal slices about 1 mm thick were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in ice-cold 0.1 M cacodylate buffer at pH 7.4. Then, cortical parts of the kidney slices were cut into 1–2 mm blocks and fixed in the same fixative for 2 hr. The tissue blocks were washed with three or four changes of 0.1M cacodylate buffer containing 8% sucrose and post-fixed in 1% OsO_4 in the same buffer for 2 hr. All fixation procedures were performed at 4°C. After dehydration in a graded series of ethanol, the tissue blocks were embedded in low viscosity 'Spurr' resin (TAAB). Ten to fifteen blocks in each rat were used for electron microscopical observations. For the identification of glomerular-tubular orientation, 0.6–1 μm thick sections were cut and stained with toluidine blue. In some blocks of old rat kidneys, the glomeruli were absent and those blocks were not used for further observation.

From the blocks which showed renal corpuscles with vascular and urinary poles in thick sections, ultrathin sections were cut with an ultramicrotome (Ultratome Nova, LKB 2188). After staining with uranyl acetate and lead citrate, sections were examined with a JOEL 1200 EX electron microscope. Glomeruli were examined and 10–20 photographs were taken at the magnification of × 8000. Semi-quantitative analyses of ultrastructural changes were carried out according to the method of Hayashida et al. [10] with some modifications. Outline of the analyses were briefly shown as follows:

(a) Thickness of GBM - In each rat, the thickness of GBM perpendicular to the basement membrane were measured at 100 points.

(b) Epithelial cells and their foot processes - Percent area of vacuoles in glomerular epithelial cell (GEC) cytoplasm was calculated in 50 GECs in a total of 400 μm^2 by point counting planimetry [11]. Size of vacuoles in each rat was randomly measured in 50 vacuoles. For the lesions of GEC foot processes, the indices of I-V as shown in Table 2 were recorded in each rat. GEC lesions such as the percent of the incidence of GEC cytoplasmic attachment to the GBM in the GECs and the cytoplasmic degeneration bearing vacuolation and slender processes were also used as parameters.

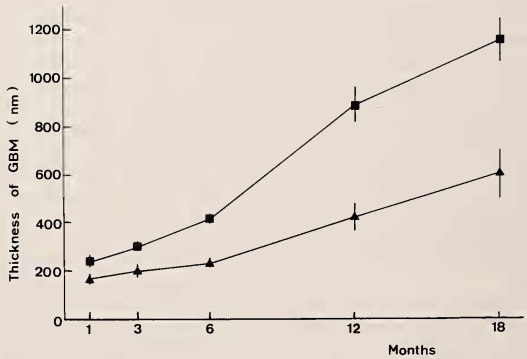

FIG. 1. Age-related changes in the thickness of GBM in male (■) and female (▲) rats. Vertical bars indicate standard errors of means (SEM). Each point depicts the mean of 5 rats.

(c) Mesangium - Indices were used for the lesions of the mesangium (Table 3).

Comparison between groups of male and female rats was made by Kruskal-Wallis test and Mann Whittney's U test.

RESULTS

Glomerular capillary basement membrane (GBM)

Age-related changes in the thickening of GBM in both sexes of rats are presented in Figure 1. The thickness of GBM increased as a function of age in both sexes. However, apparent sex difference was present. At 12 and 18 months of age, the GBM of male rats was about twice thicker than that of female rats ($P<0.005$). The frequency of ultrastructural lesions in the GBM is shown in Table 1. Typical ultrastructural changes occurring in the GBM are shown in Figure 2. Two rats with focal thickening of lamina densa with three distinct layers and two other rats with segmentally thickened GBM without three distinct layers were found at 6 months. At 18 months of age all male rats showed extensive thickening with nodular foldings of GBM. However, female rats started showing focal thickening of lamina densa without three distinct layers of GBM at 12 months. Only at 18 months female rats showed obvious thickening of GBM (Fig. 3a).

Glomerular epithelial cell (GEC)

Morphological changes of GEC cytoplasm include the increase in the area of vacuoles, the size of vacuoles, the attachement of GEC cytoplasm to the GBM and the degeneration of GEC cytoplasm (Figs. 4–6). The most widespread changes in both sexes of rats were the vacuolation in the rough endoplasmic reticulum in GEC cytoplasm (Figs. 3b and 7). The highest percentage and the biggest diameter of vacuoles were found in male rats at 12 months of age. However, 18-month-old male rats showed lower percentage of vacuoles with smaller diameter than 12-month-old ones (Figs. 7b and 8). In addition, the attachment of GEC cytoplasm to the GBM was observed in about 70 and 80% of the GECs in 12- and 18-month-old male rats, respectively (Fig. 6). Eighteen-month-old male rats showed degeneration of GEC cytoplasm in about 70% of the cells (Fig. 7b), while in female rats it was about 3% (Fig. 3 and 6).

Glomerular epithelial cell foot processes

Age-related changes of GEC foot process lesions were recorded as indices from I-V (Fig. 9) and the incidence of lesions in GEC foot processes in male and female rats is shown in Table 2. Frequent occurrence of focal and scattered fusion

Table 1. Frequency of ultrastructural lesions in GBM in male and female rats of various ages

Sex	Age in months	No. of rats	Normal	Lesion				
				I	II	III	IV	V
Male	1	5	4	1	—	—	—	—
	3	5	—	3	2	—	—	—
	6	5	—	1	2	2	—	—
	12	5	—	—	—	3	1	1
	18	5	—	—	—	—	1	4
Female	1	5	5	—	—	—	—	—
	3	5	4	1	—	—	—	—
	6	5	2	2	1	—	—	—
	12	5	—	1	2	2	—	—
	18	5	—	—	1	4	—	—

Lesion indices: I. Absence of significant ultrastructural abnormalities except focal and scattered thickening of lamina densa with distinct three layers. II. Focal thickening of lamina densa with distinct three layers. III. Segmental thickening of GBM without distinct three layers. IV. Thickening and folding of GBM. V. Extreme thickening and nodular folding of GBM.

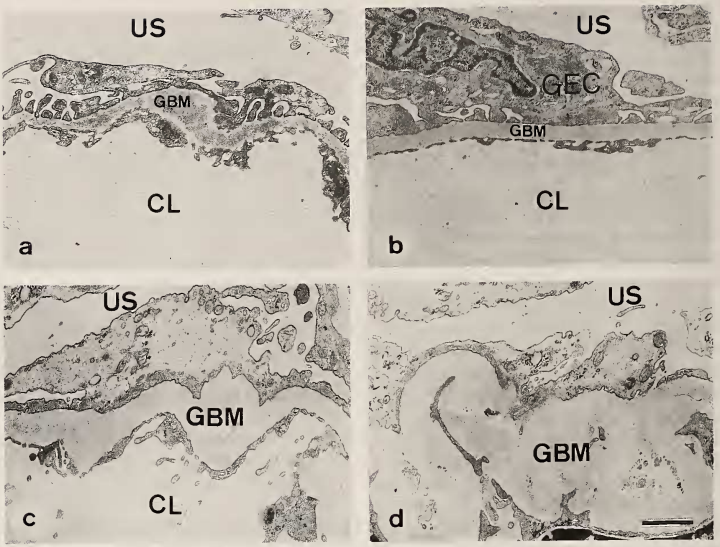

FIG. 2. Electron microphotographs illustrating lesions of II-V (refer to Table 1 for indices) in glomerular basement membrane (GBM). Bar: 1μm. CL, capillary lumen; GEC, epithelial cell; US, urinary space.
a. Focal thickening of lamina densa with three distinct layers (Lesion II).
b. Thickening of GBM without three distinct layers (Lesion III).
c. Thickening and folding of GBM (Lesion IV).
d. Extreme thickening and nodular foldings of GBM (Lesion V).

of GEC foot processes were apparent at 3 months of age in male rats and total degeneration of GEC developed at 18 months in 2 out of 5 male rats. However, in female rats no severe changes were observed, except focal and segmental flattening of fused foot processes at 12 months of age.

Mesangium

Increase in amount of the mesangial matrix in male and female rats is shown in Table 3. Frequent occurrence of fusion of the mesangial matrix to the GBM was apparent in 6- and 12-month-old male and 12- and 18-month-old female rats. However, 2 out of 5 males at 12 months of age and all 18-month-old male rats showed massive mesangial matrix. The massive mesangial matrix filled the glomerular capillary lumen which resulted in the obliteration of the lumen (Fig. 8a). In 18-month-old male rats the mesangial cell cytoplasm underwent shrinkage due to massive accumulation of the mesangial matrix (Fig. 8b).

Endothelial cell cytoplasm

One-, 3- and 6-month-old male rats and all female rats showed normal structure of the endothelial cell cytoplasm (Fig. 3). However, in 12- and 18-month-old male rats the numbers of thin layers of the endothelial cytoplasm and the fenestrae were reduced (Fig. 8).

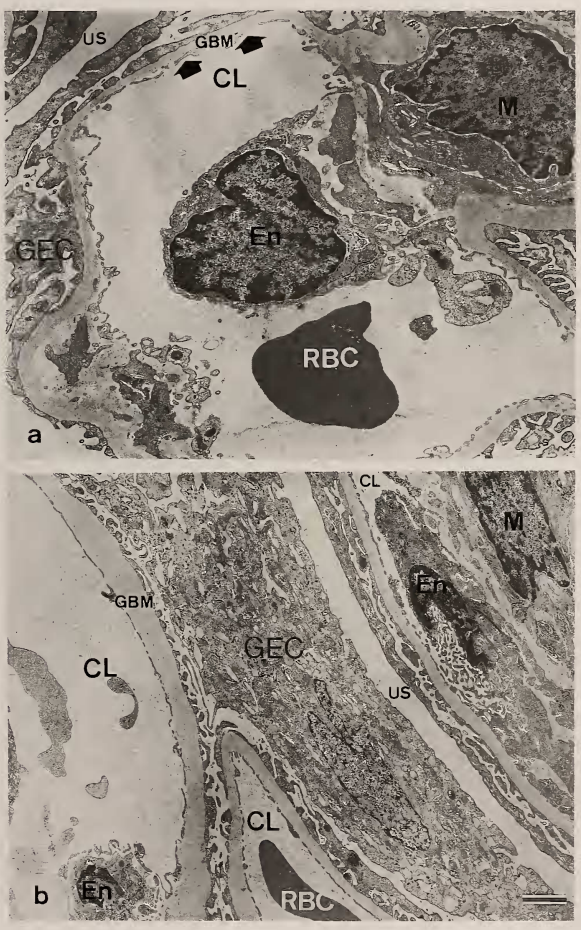

Fig. 3. Electron microphotographs of a part of glomerulus of an 18-month-old female rat. Bar: 1 μm. CL, capillary lumen; En, endothelial cell; GBM, glomerular basement membrane; GEC, epithelial cell; M, mesangial cell; RBC, red blood cell; US, urinary space.

a. Thickening of GBM with focal fusion of epithelial cell foot processes (arrows). Mesangial cell and endothelial cell show no significant abnormalities.

b. Vacuolation of the rough endoplasmic reticulum in the cytoplasm of glomerular epithelial cell.

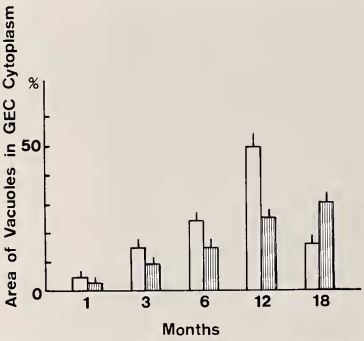

FIG. 4. Age-related changes in % area of vacuoles in GEC (glomerular epithelial cell) cytoplasm in male (open columns) and female (hatched columns) rats. Vertical bars indicate SEM. Each point depicts the mean of 5 rats.

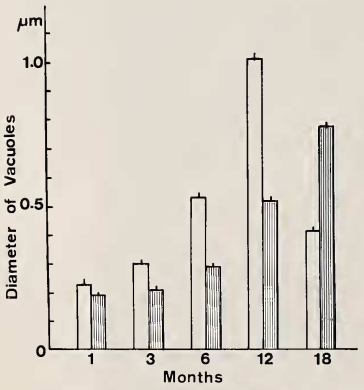

FIG. 5. Age-related changes in diameter of vacuoles in male (open columns) and female (hatched columns) rats. Each column shows the mean and SEM of 5 rats.

DISCUSSION

We have recently reported that the earliest ultrastructural changes of the renal glomerulus

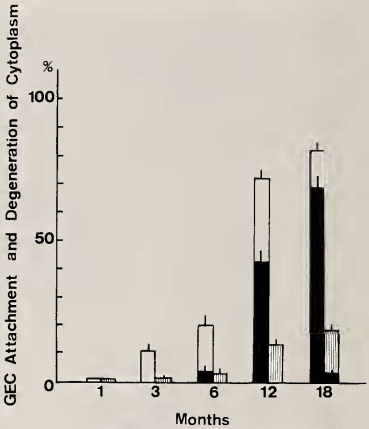

FIG. 6. Age-related changes of % GEC attachment to the GBM and degeneration of cytoplasm in male (open columns) and female (hatched columns) rats. The black columns indicate the degeneration of GEC cytoplasm. Each column shows the mean and SEM of 5 rats.

were observed in male Wistar/Tw rats at the age of 3 months. The changes were slight thickening of GBM, blurring of lamina densa and focal fusion of epithelial cell foot processes [7]. Similar morphological changes have been observed in Sprague-Dawley rats [8] and Wistar rats [9] at 6 months of age. Age-related changes in the kidney are characterized by the thickening of GBM [9, 10, 12] which was confirmed in our recent paper [7]. However, in Wistar/Tw male rats the thickness of GBM was about twice greater than in female rats at 12 and 18 months of age.

Kurtz and Feldman [13] and Walker [14] suggested that the epithelial cell foot processes manufacture a major proportion of GBM material and the changes of the GEC and GBM is interrelated [15]. Several authors have reported that GEC injury is associated with a number of experimental and clinical glomerular disease [16–21]. The age-related changes that occurred in the GBM and GEC and their foot processes were closely interrelated each other in Wistar/Tw rats and the changes were sexually different. Only male rats showed

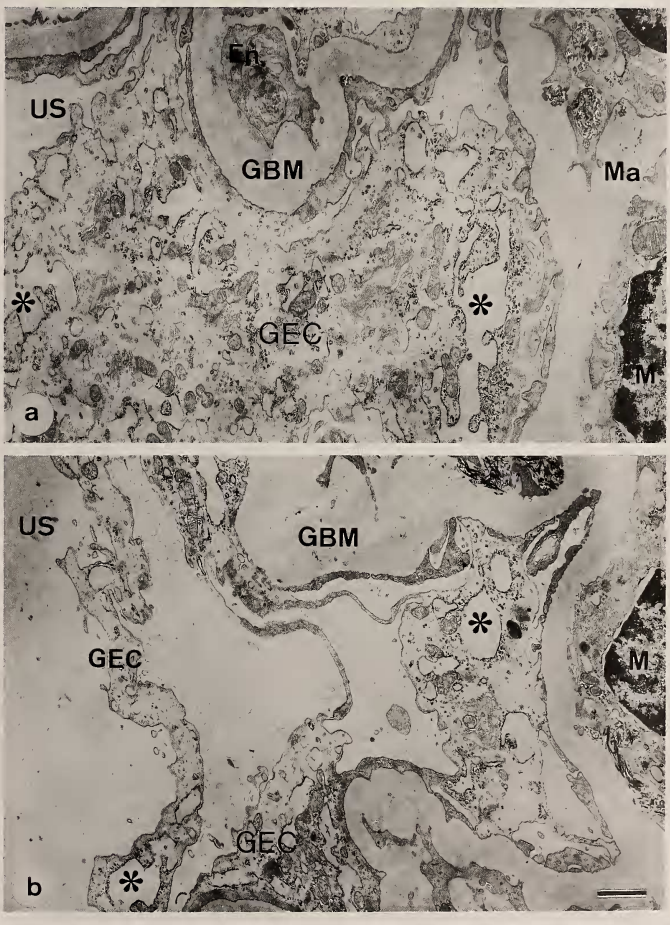

FIG. 7. Electron microphotographs of a part of glomerulus of an 18-month-old male rat. Bar: 1 μm. En, endothelial cell; GBM, glomerular basement membrane; GEC, epithelial cell; M, mesangial cell, Ma, mesangial matrix; US, urinary space.

a. The cytoplasm of glomerular epithelial cell containing vacuoles of rough endoplasmic recticulum (asterisks).

b. Attachment of epithelial cell cytoplasm to GBM and degeneration with vacuoles (asterisks).

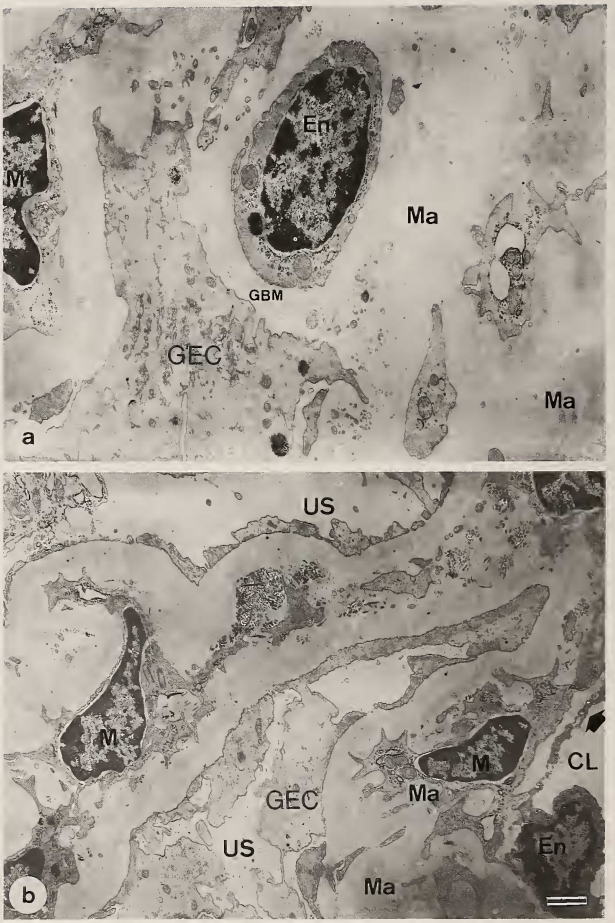

FIG. 8. Electron microphotographs of a part of the glomerulus of male rats at 12 (a) and 18 months (b) of age. Bar: 1 μm. CL, capillary lumen; En, endothelial cell; GBM, glomerular basement membrane; GEC, epithelial cell; M, mesangial cell; Ma, mesangial matrix; US, urinary space.

a. Glomerular capillary lumen is filled with massive mesangium matrix and the endothelial cell is shrunken. Note disappearance of thin layer cytoplasm of the endothelial cell.

b. Mesangial and endothelial cell cytoplasm is shrunken by massive mesangium matrix. Note reduction of thin layer cytoplasm and fenestrae of the endothelial cell (arrow).

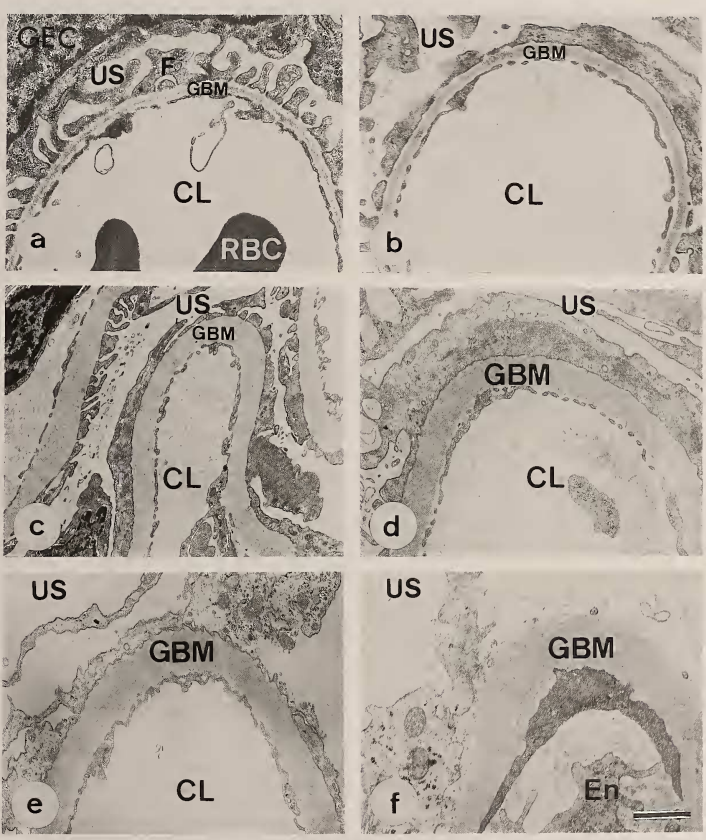

FIG. 9. Electron microphotographs illustrating lesions in the glomerular epithelial cell (GEC) foot processes. Bar: 1 μm. CL, capillary lumen; En, endothelial cell; F, foot process; GBM, glomerular basement membrane; GEC, epithelial cell; RBC, red blood cell; US, urinary space.

a. Normal GEC foot processes.
b. Lesion I: focal and scattered fusion of GEC foot processes.
c. Lesion II: segmental flattening and extensive fusion of GEC foot processes.
d. Lesion III: zonal fusion of GEC foot processes and attachment of GEC cytoplasm to GBM.
e. Lesion IV: modest degeneration of GEC foot processes.
f. Lesion V: total degeneration of GEC foot processes.

TABLE 2. Age-related changes in incidence of lesions in the glomerular epithelial cell (GEC) foot processes in male and female rats

Sex	Age in months	No. of rats	Normal	Lesion				
				I	II	III	IV	V
Male	1	5	4	1	—	—	—	—
	3	5	—	5	—	—	—	—
	6	5	—	1	4	—	—	—
	12	5	—	—	—	3	2	—
	18	5	—	—	—	1	2	2
Female	1	5	5	—	—	—	—	—
	3	5	4	1	—	—	—	—
	6	5	2	3	—	—	—	—
	12	5	—	2	3	—	—	—
	18	5	—	—	4	1	—	—

Lesion indices: I. Frequent occurrence of focal and scattered fusion of foot processes. II. Segmental flattening and extensive fusion of GEC foot processes. III. Zonal fusion of GEC foot processes and attachment of GEC cytoplasm to GBM. IV. Modest degenerative changes of GEC foot processes. V. Total degeneration of GEC foot processes.

TABLE 3. Age-related changes in mesangium in male and female rats

Sex	Age in months	No. of rats	Normal	Lesion			
				I	II	III	IV
Male	1	5	5	—	—	—	—
	3	5	—	4	1	—	—
	6	5	—	1	2	2	—
	12	5	—	—	—	3	2
	18	5	—	—	—	—	5
Female	1	5	5	—	—	—	—
	3	5	4	1	—	—	—
	6	5	2	2	1	—	—
	12	5	—	1	3	1	—
	18	5	—	—	1	4	—

Lesions indices: I. Focal occurrence of fusion of mesangial matrix to GBM. II. Frequent occurrence of fusion of mesangial matrix to GBM. III. Frequent occurrence of fusion of mesangial matrix to GBM with some accumulation of mesangial matrix. IV. Very frequent occurrence of fusion of mesangial matrix to GBM with massive accumulation of mesangial matrix.

huge vacuolation at 12 months with the attachment of GEC cytoplasm to the GBM and some 12-month-old and all 18-month-old rats showed the degeneration of GEC cytoplasm.

Enlargement of mesangium was frequently observed as age-related changes [8, 10]. However, Gray *et al.* [22] reported that there was no widening of the mesangial area in aged Sprague-Dawley rats. In the present study there was frequent occurrence of fusion of mesangial matrix to the GBM in both sexes which increased with age. Massive enlargement of mesangium was encountered only at 12- and 18-month-old male rats.

Kreisberg and Karnovsky [21] suggested that persistent injury to the epithelial cells could lead to the alteration in glomerular permeability which resulted in the accumulation of proteins in the mesangial area and could consequently lead to mesangial cell injury. In Wistar/Tw rats, while GEC cytoplasm started to degenerate at 12 months of age, huge mesangium accumulation occurred at more advanced ages. These findings support the view of Kreisberg and Karnovsky [21]. In addition, we found the reduction and disappearance of fenestrae and thin cytoplasm layer of endothelial cells at 12 and 18 months of age in male rats.

To conclude, the results of the present study indicate that the epithelial cell injury could lead not only to mesangial cell injury but also to endothelial cell injury, and that there is a clear sex difference in the age-related changes of the renal glomerulus at 12 and 18 months of age. In order to know the cause of this sex difference, the study on the influence of androgen on these changes is under way.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (Nos. 62480023 and 63304008 to S.K.).

REFERENCES

1 Kobayashi, Y. and Kawashima, S. (1980) Polydipsia and polyuria in aged male rats of the Wistar/Tw strain. Proc. Japan Acad., Ser. B., **56**: 643–648.

2 Kobayashi, Y. and Kawashima, S. (1984) Age-related changes in the water and electrolyte metabolism in male rats of the Wistar/Tw strain. Exp. Gerontol., **19**: 107–113.

3 Kawashima, S. and Kobayashi, Y. (1982) Morphometric study of the hypothalamo-neurohypophyseal system in aged rats. J. Sci. Hiroshima Univ. Ser. B., Div. 1, **30**: 229–242.

4 Kobayashi, Y. and Kawashima, S. (1983) Histological changes in the kidney of aged rats of the Wistar/Tw strain showing polydipsia and polyuria. J. Sci. Hiroshima Univ., Ser. B., Div. 1, **31**: 149–154.

5 Win Win Yee and Kawashima, S. (1987) Sex difference in the early histopathological changes of the kidney in Wistar/Tw rats. Zool. Sci., **4**: 867–873.

6 Kawashima, S., Kawamoto, K. and Kobayashi, Y. (1986) Aging of the hypothalamo-neurohypophysial system and water metabolism in rats. Zool. Sci., **3**: 227–244.

7 Win Win Yee, Takahashi, S. and Kawashima, S. (1987) An ultrastructural study of renal glomerular capillaries and collecting tubules in aged male rats of the Wistar/Tw strain. J. Sci. Hiroshima Univ., Ser. B., Div. 1, **33**: 1–9.

8 Couser, W. G. and Stilmant, M. M. (1975) Mesangial lesions and focal glomerular sclerosis in the aging rat. Lab. Invest., **33**: 491–501.

9 Hirokawa, K. (1975) Characterization of age-associated kidney disease in Wistar rats. Mech. Ageing Dev., **4**: 301–316.

10 Hayashida, M., Yu, B. P., Masoro, E. J., Iwasaki, K. and Ikeda, T. (1986) An electron microscopic examination of age-related changes in the rat kidney: the influence of diet. Exp. Gerontol., **21**: 535–553.

11 Weibel, E. R. (1969) Stereological principles for morphometry in electron microscopic cytology. Int. Rev. Cytol., **26**: 235–302.

12 Anderson, S. and Brenner, B. M. (1986) Effects of aging on the renal glomerulus. Am. J. Med., **80**: 435–442.

13 Kurtz, S. M. and Feldman, J. D. (1962) Experimental studies on the formation of glomerular basement membrane. J. Ultrastruct. Res., **6**: 19–27.

14 Walker, F. (1973) The origin, turnover and removal of glomerular basement membrane. J. Path., **110**: 233–244.

15 Venkatachalam, M. A., Cotran, R. S. and Karnovsky, M. J. (1970) An ultrastructural study of glomerular permeability in aminonucleoside nephrosis using catalase as a tracer protein. J. Exp. Med., **132**: 1168–1180.

16 Farquhar, M. G. and Palade, G. E. (1961) Glomerular permeability. II Ferritin transfer across the glomerular capillary wall in nephrotic rats. J. Exp. Med., **114**: 699–715.

17 Venkatachalam, M. A., Karnovsky, M. J. and Cotran, R. S. (1969) Glomerular permeability: Ultrastructural studies in experimental nephrosis using horseradish peroxidase as a tracer. J. Exp. Med., **130**: 381–399.

18 Messina, A., Davies, D. J., Dillane, P. C. and Ryan, G. B. (1987) Glomerular epithelial abnormalities associated with the onset of proteinuria in aminonucleoside nephrosis. Am. J. Path., **126**: 220–229.

19 Ryan, G. B. and Karnovsky, M. J. (1975) An ultrastructural study of the mechanisms of proteinuria in aminonucleoside nephrosis. Kidney Int., **8**: 219–232.

20 Kanwar, Y. S. and Rosenzweig, L. J. (1982) Altered glomerular permeability as a result of focal

detachment of the visceral epithelium. Kidney Int., **21**: 565–574.

21 Kreisberg, J. I. and Karnovsky, M. J. (1978) Focal glomerular sclerosis in the Fawn-Hooded rat. Am. J. Path., **92**: 637–652.

22 Gray, J. E., Weaver, R. N. and Purmalis, A. (1974) Ultrastructural observations of chronic progressive nephrosis in the Sprague-Dawley rat. Vet. Path., **11**: 153–164.